Proceedings of ANAS (Biological and Medical Sciences), vol. 73, No 2, p. 19-24 (2018)

Reactivation of the Oxygen-Evolving Function of Photosystem II Inhibited by Hydroxylamine

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The oxygen-evolving activity of photosystem II (PSII) was inhibited by extraction of the Mn-cluster using hydroxylamine. The activity was recovered partially by the photo-activation treatment applied after addition of the exogenous Mn^{2+} to the inhibited samples. The effect of Ca^{2+}, Cl^- and H^+ ions in the reaction medium on reconstitution of the oxygen-evolving activity of PSII was studied. The results can be useful for the understanding of the principles of artificial energy converters based on photosynthetic reaction centers and for the study of artificial photosynthesis.

Keywords: *Photosystem II, molecular oxygen, hydroxylamine, Mn-cluster, calcium, chloride, pH*

INTRODUCTION

Photosystem II (PSII) involved in the energy conversion in thylakoid membranes of oxygenic species, catalyzes a very important biological function – oxidation of water molecules and formation of molecular oxygen (Barber, 2006; Muh and Zouni, 2011). Structure of its photochemical core determined with high accuracy in cyanobacteria and red algae (Zouni et al., 2001; Ferreira et al., 2004; Umena et al. 2011; Ago et al., 2016). This molecular complex is composed of ~20 proteins and numerous cofactors. Among them transmembrane D_1 and D_2 proteins (32-34 kDa), cytochrome b_{559}, core antenna proteins CP47 and CP43 carrying Chl *a*, and peripheral proteins of 33-, 24- and 18 kDa located on the lumen surface of thylakoid membranes are the most important. D_1/D_2 heterodimer of the PSII complex consists of an initial electron donor P_{680} (chlorophyll *a* dimer), electron acceptors pheophytin (Phe) and plastoquinones (O_A, Q_B), redox active tyrosines Y_Z (D_1-Tyr^{161}) and Y_D (D_2-Tyr^{161}) (Barber, 2006; Muh and Zouni, 2011). The light absorption in the reaction center results in the electron transport from P_{680} dimer to Phe molecule and then consistently to plastoquinones Q_A and Q_B. The oxidized form of the initial electron donor P_{680} ($P_{680}^{\bullet+}$) is a strong oxidant (~1.1 V), which is eventually reduced at the expense of electrons transported from the water oxidation center (Klimov et al. 1977; Klimov and Krasnovski, 1981). The core of the water oxidation center composed of the Mn_4CaO_5 cluster. Tyrosine Y_Z residue of D_1 protein participates in the electron transfer between the Mn_4CaO_5 cluster and $P_{680}^{\bullet+}$.

When the water molecule is oxidized, the complex PSII passes through the states S_0, S_1, ..., S_4 of the hypothetical S-cycle. Formation of molecular oxygen occurs in the final $S_3 \rightarrow [S_4] \rightarrow S_0$ transition. It is assumed that the positive charges produced in the S-cycle, accumulate in the Mn_4CaO_5 cluster (Kok et al., 1970; Debus, 1992; Muh and Zouni, 2011). PSII kept in darkness for a long time is stabilized mainly at S_1 and S_0 (75% and 25%, respectively) states. S_0 and S_1 states are, respectively, semi-stable and stable, whereas S_2 and S_3 are energetically unstable. They return to the S_1 state in the dark. S_4 state is a transitional, and spontaneously passes to S_0. Molecular oxygen is formed in the S_3-S_4-S_0 transition and released to atmosphere (Debus, 1992; Muh and Zouni, 2011).

Recently, intensive investigations have been carried out on developing various biomimetic devices including artificial energy converters on the basis of the structure and functional principles of photosynthetic complexes (reviewed in: Barber, 2009; McConnell et al., 2010; Barber and Tran, 2013). Due to effective energy convertion (quantum yield of primary reactions of photosynthesis is ~1.0) and high oxidation potential PSII is considered to be useful for these investigations. A main goal of the investigations of artificial systems is the elucidation of principles of the assembly of components (antenna, reaction center, components of the oxygen-evolving machinery etc.) of energy converters (Hamarstrom and Styring, 2008; Brudvig, 2008; Dismukes et al., 2009; Kanady et al., 2011). Consequently, the study of the photosynthetic processes by inhibition and recovery of the reactions occurring in the natural system and

the role of various components in the natural phytosystems becomes important.

In our research the oxygen-evolving function of the water oxidation center was inhibited by extraction of the Mn_4CaO_5 cluster from the PSII complex. Then, this function was restored through photoactivation by adding exogenous Mn^{2+}. The effect of various factors such as Ca^{2+}, Cl^{-}, pH on effective reconstitution of the oxygen-evolving activity has been studied.

MATERIALS AND METHODS

PSII membrane fragments (BBY type) having a high oxygen-evolving capacity (~600 μmol O_2 (mg chl)$^{-1}$s^{-1}) were isolated from spinach, using Triton X-100 (Berthold et al., 1981; Völker et al. 1985). For extraction of the Mn_4CaO_5 cluster, PSII membranes were homogenized in 25 mM MES-NaOH (pH 6.5) buffer containing 400 mM sucrose, 20 mM NaCl (buffer A) at chlorophyll concentration ~0.5 mg/ml. After addition of hydroxylamine (NH_2OH), incubation was performed in darkness at room temperature (~25°C). At the end of incubation the homogenate was diluted 5-6 times with the cold (~4°C) buffer A and centrifuged at 18,000 g, at 4°C. The precipitate was washed 3 times through the homogenization in the cold buffer A and precipitation at 18,000 g, at 4°C. Reconstruction of the oxygen-evolving activity of PSII was performed in the buffer A, containing $MnCl_2$ at room temperature, under photoactivating light (~50 μmol photon m^{-2}s^{-1}). The same method was used to study the effect of various factors on photoactivation. The chlorophyll content was evaluated through spectrophotometric (JY-201, Jobin Ivon, France) determination of optical density of its 80% acetone extract at 663 nm and 645 nm (MacKinney, 1941). The rate of O_2 evolution of the PSII complex was measured amperometrically using the Clark electrode (Rank Brothers Ltd., UK).

RESULTS AND DISCUSSION

Hydroxylamine added PSII membranes lose their Mn cluster very rapidly. This treatment results in almost complete inhibition of O_2 evolution and electron transport activities of the PSII complex. The inhibitory effect of hydroxylamine depends on its concentration, content and temperature of the incubation medium. It is known that preparations treated with hydroxylamine are more sensitive to light inhibition compared with native PSII complexes. Reactivation of O_2 evolution and electron transport activities of Mn-depleted preparations are still an object of discussions. The effect of 1.0-3.0 mM hydroxylamine on the oxygen-evolving activity of PSII membranes has been examined in the presented research. As seen in figure 1, inhibition of the oxygen yield of PSII membranes was very fast during the first 5 minutes of incubation. Subsequent (>5 min) incubation with hydroxylamine demonstrates only slow inhibition. Consequently, the optimal effective concentration of hydroxilamine was found to be 2.0 mM and duration of effective action was 5 min.

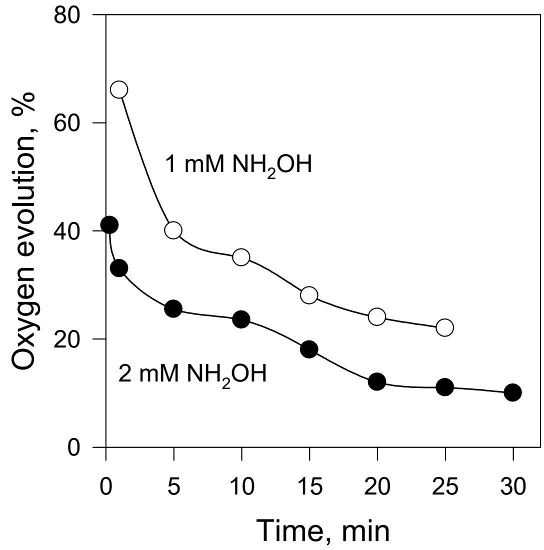


Fig. 1. The change in the oxygen evolution activity of the PSII membrane preparations in the presence of hydroxylamine. The maximum oxygen yield of the native preparations (600 μmol O_2 (mg chl)$^{-1}$s^{-1}) was assumed to be 100%. During the incubation with hydroxylamine the ambient temperature was 25°C, and chlorophyll concentration was 0.5 mg/ml. For the incubation and measurements 25mM MES-NaOH (pH 6.5) buffer, containing 400 mM sucrose and 20 mM NaCl was used.

Reactivation of the oxygen evolution activity through reassembling the Mn-cluster in inhibited PSII preparations, and its dependence on the pH, concentrations of Ca^{2+} and Cl^{-} ions have been studied. The oxygen-evolving activity of the inhibited PSII complex in preparations with the extracted Mn-cluster was partly restored through photoactivation (~20-35% in various preparations) after addition of exogenous Mn^{2+} ($MnCl_2$). The necessity of adding 1-3 mM Mn^{2+} into the reaction medium for restoration of the oxygen-evolving activity was established (Fig. 2). Maximum restoration of the oxygen-evolving function was ensured by 10-15 min illuminatuion with ~50 μmol photon m^{-2}s^{-1} white light.

Ca^{2+} is involved in the water oxidation center (Debus, 1992; Vrettos et al., 2001; Ugur et al., 2016).

The necessity of the presence of high Ca^{2+} concentrations in the reaction medium for effective reconstruction of the Mn-cluster was reported previously (Ghanotakis et al., 1984; Miller and Brudvig, 1989; Chen et al. 1995). We also established that to provide high yield oxygen evolution addition of 20-50 mM Ca^{2+} ($CaCl_2$) to the reaction medium is required. At higher concentrations of Ca^{2+} ions (>50 mM) an additional increase in the oxygen-evolving activity of reconstituted PSII preparations was not observed (Fig. 3). However, the amount of Ca^{2+} ions for restoration of the oxygen-evolving activity is much more higher compared with the native PSII (~1 Ca^{2+}/RC).

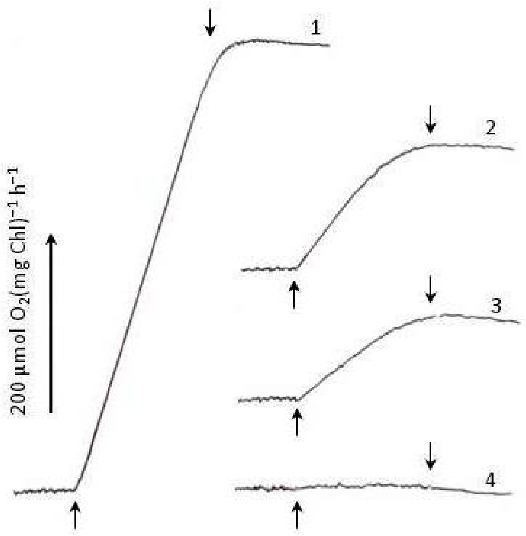


Fig. 2. Oxygen evolution in PSII particles inhibited by hydroxylamine and repaired by photoactivation: 1 – native, 2 – treated with hydroxylamine, 3 – in the presence of 2 mM $MnCl_2$, and 4 – kinetics of oxygen evolution in the photoactivated PSII preparations in the presence of 2 mM $MnCl_2$ and 20 mM $CaCl_2$. The medium 25mM MES-NaOH (pH 6.5), containing 400 mM sucrose and 20 mM NaCl was used for photoactivation and O_2-measurements Fotoactivation was performed at room temperature for 10 min, under illumination of ~50 μmol photon $m^{-2}s^{-1}$ white light. ↑ (↓) – measutring light on (off).

The presence of Cl^- ions is also required for the maximum oxygen-evolving activity of PSII (Sandusky and Yocum, 1986; Miyao and Inoue, 1991, Boussac and Rutherford, 1994; Olesen and Andreasson, 2003). However, in our experiments, high yield of oxygen evolution achieved after photoactivation treatment, required much higher concentration of Cl^- ions (≥1.0 M) compared with Ca^{2+} ions (Fig. 4). This fact may be attributed to the lower affinity of Cl^- ions to the water oxidation center compared with Ca^{2+} ions.

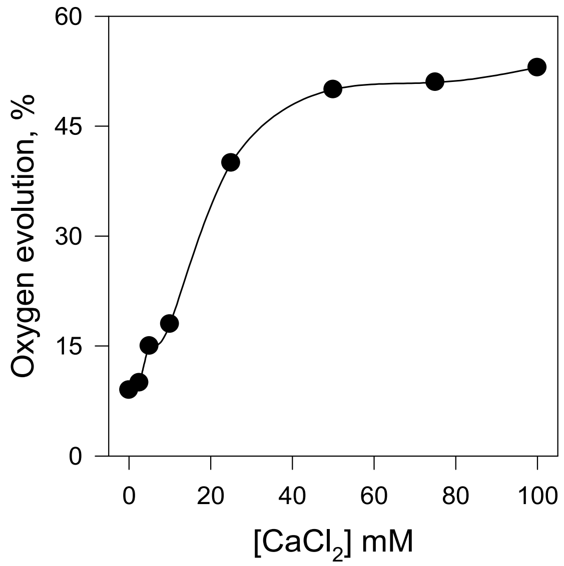


Fig. 3. The effect of Ca^{2+} ions on reactivation of the oxygen-evolving activity of PSII. The maximum oxygen yield (600 μmol O_2 (mg chl)$^{-1}$s^{-1}) was assumed to be 100%. The medium used for photoactivation and measurements as described in Fig. 2. In addition, photoactivation medium includes 2 mM $MnCl_2$. Photoactivation was performed at room temperature for 10 min, under illumination of ~50 μmol photon $m^{-2}s^{-1}$ white light.

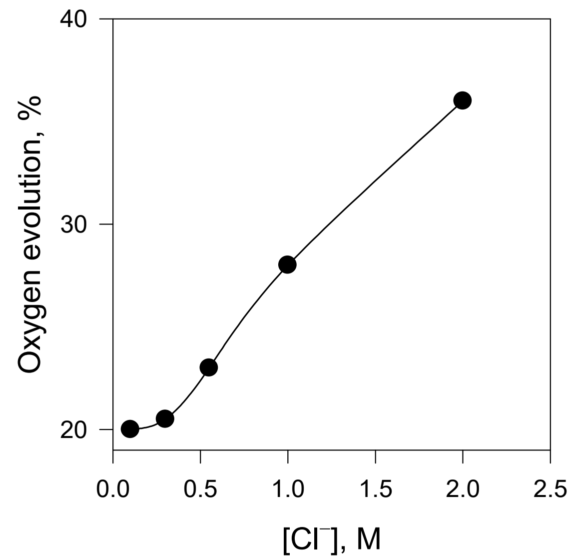


Fig. 4. The effect of Cl^- ions on reconstitution of the oxygen-evolving activity of the PSII complex. The medium used for photoactivation and measurements as described in Fig. 2. In addition, photoactivation medium includes 2 mM $MnCl_2$ and 20 mM $CaCl_2$. Fotoactivation was performed at room temperature for 10 min, under illumination of ~50 μmol photon $m^{-2}s^{-1}$ white light.

Considering importance of pH value for O_2-evolution, we studied reconstruction of the Mn-cluster in the pH range from 5.5 to 6.5. According to the results of our experiments, oxygen evolution enhanced with increasing pH (from 5.5 to 6.5). In other words, the pH-dependent change of the

restored value of the oxygen yield occurred in the following direction: pH 5.5<5.9<6.2<6.5 (Fig. 5). Photoactivation of O_2 evolution at higher values of pH was not studied, as higher values of pH were assumed to inhibit the oxygen-evolving complex.

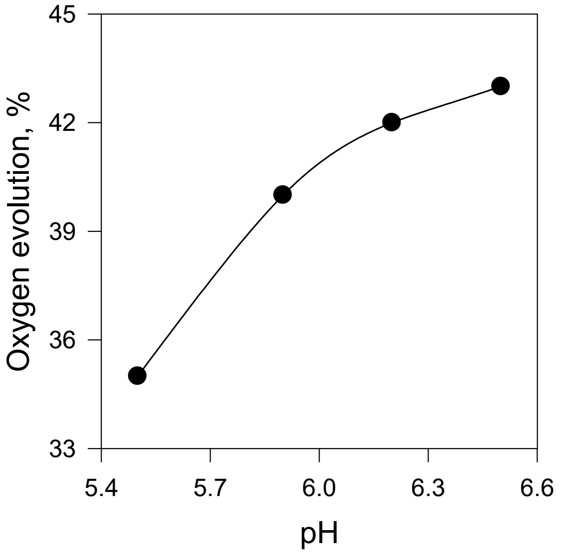


Fig. 5. The effect of pH of the reaction medium on restoration of the oxygen-evolving activity of the PSII complex. The medium used for photoactivation and measurements as described in Fig. 2. In addition, photoactivation medium includes 2 mM $MnCl_2$ and 20 mM $CaCl_2$. Fotoactivation was performed at room temperature for 10 min, under illumination of ~50 μmol photon $m^{-2}s^{-1}$ white light.

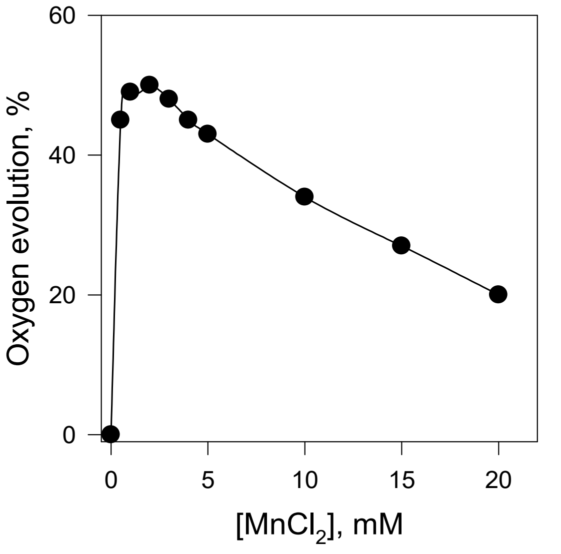


Fig. 6. Reconstruction of the oxygen-evolving activity of the PSII complex in dependence of concentrations of Mn^{2+} ions in the photoactivation medium. The medium used for photoactivation and measurements as described in Fig. 2. Besides Mn^{2+}, the photoactivation medium contains 20 mM $CaCl_2$ and ~100 mM Cl^- ions. Photoactivation was performed at 25°C, for 10 min, at light intensity 50 μmol photon $m^{-2}s^{-1}$.

Figure 6 presents the the oxygen evolving activity of PSII photoactivated at different concentrations of Mn^{2+} in the presence of 20 mM Ca^{2+} and 100 mM Cl^- ions, and optimum pH value of 6.5. As seen in the figure, the oxygen yield was higher at 1-5 mM concentrations of Mn ions and declined with increasing concentrations of these ions.

Thus, the partly restoration of the oxygen-evolving function of PSII inhibited by hydroxylamine has been established as a result of the experimental evaluation of the possibility of reconstruction of the PSII preparations with the extracted Mn-cluster.

Considering all the optimization mechanisms, maximum yield of oxygen evolution in the reconstituted PSII was less than 50% of that observed in the native complex.

Possible reasons for such a big difference in the oxygen evolving activity of the reconstructed and native complexes, observed in our experiments, may include: (i) initiation of the other inhibitory mechanisms (for example, removal of peripheral proteins from the binding sites, photoinhibition etc.) during the preparation process; (ii) incomplete reassembling of the Mn cluster; (iii) high concentrations of the ions in the reaction medium, required for the reversibility of oxygen evolution, and thereby possibility of the both activatory and inhibitory effects of these ions on the PSII electron transport. On the other hand, a very high value of Mn^{2+}/RC stoichiometry (~10^4 times compared with the native complex) for maximum photoactivation of the oxygen yield, also may be questioned.

Currently, the research on the identification of the inhibition mechanisms and optimization of restoration of the oxygen-evolving complex continues.

ACKNOWLEDGMENTS

This work was supported by the Science Development Foundation under the President of the Republic of Azerbaijan – **Grant № EIF-BGM-4-RFTF-1/2017-21/20/3**, and by the Presidium of Azerbaijan National Academy of Sciences – **Grant dated on 15.03.2017**.

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Hidroksilaminlə İnhibirləşmiş Fotosistem II Kompleksinin Oksigen Ayırma Funksiyasının Bərpası

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Fotosistem II (FS II) kompleksində, suyun oksidləşdiyi katalitik mərkəzin Mn-klasteri hidroksilaminlə ekstraksiya olunaraq, oksigen ayırma fəallığı inhibirə edilmişdir. Ekzogen Mn^{2+} əlavə olunduqda inhibirə olunmuş kompleksin oksigen ayırma fəallığı foto-aktivləşmə yolu ilə qismən bərpa olunmuşdur. Reaksiya mühitindəki Ca^{2+}, Cl^{-} və H^{+} ionlarının miqdarının, bərpa prosesinə təsiri öyrənilmişdir. Alınmış nəticələr FSII-nin iş prinsiplərinə əsaslanan süni enerji çeviricilərinin işlənib hazırlanması və süni fotosintez tədqiqatları üçün əhəmiyyətli ola bilər.

Açar sözlər: *Fotosistem II, molekulyar oksigen, hidroksilamin, Mn klasteri, kalsium, xlorid, pH*

Восстановление Кислородвыделяющей Функции Фотосистемы 2, Ингибированной Гидроксиламином

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Выделение кислорода комплексом фотосистемы 2 (ФС-2) ингибировалось после экстракции Mn-кластера центра окисления воды. После добавления экзогенного Mn^{2+}, активность выделения кислорода ингибированной ФС-2 частично восстанавливалась в процессе фотоактивации. Изучено влияние ионов Ca^{2+}, Cl^{-} и H^{+}, присутствующих в реакционной среде, на восстановление кислородвыделяющей активности ФС-2. Результаты могут быть полезными для разработки искуственных преобразователей энергии на основе принципов функционирования ФС-2 и для исследования искусственного фотосинтеза.

Ключевые слова: *Фотосистема 2, молекулярный кислород, гидроксиламин, марганцевый кластер, кальций, хлор, pH*